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Research Article

IN SILICO ANALYSIS OF A PUTATIVE MOLYBDENUM TRANSPORTER (MoT) IN BUFFALO

Santanu Panda[1*], Jai Kumar Kaushik[2], Ashok Kumar Mohanty[2]

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ABSTRACT: Almost all living organism require to transport molybdenum (Mo) from outside cellular milieu for maintenance of essential life processes. Mo is serving as cofactor for catalytic activity of xanthine dehydrogenase, aldehyde oxidase and sulphite oxidase from bacteria to mammals. Although Mo transport is well established in prokaryotes and some eukaryotes including plants, little information is available on Mo transport system in mammals. Recently, HsMoT2/MFSD5 was reported to transport Mo by its over expression in HEK-293T cells. But the structural and functional annotation of MoT2/MFSD5 gene product is missing in mammals and still no sequence information is available on farm animals, particularly in buffalo. Here we cloned and sequenced buffalo MoT/MFSD5 and analysed the sequence *in silico*. We predicted that buffalo MoT is unstable, hydrophobic and trans membrane protein with 50KD molecular weight. It is having 11 trans membrane helices to traverse the cell membrane. Functionally the protein shared sequence homology with a sugar porter family, bovine MFSD5. Three-dimensional model of buffalo MoT was also analysed using Raptor X server that predicted the protein is having two-fold pseudo symmetry between N and C terminal domain. Finally, we performed comparative analysis of buffalo MoT with human and cattle which revealed the protein is highly conserved both structurally and functionally. Thus, the structural and functional annotation of buffalo MoT would pave a way to design suitable mutants or inhibitors which could be exploited to manage Mo deficiency and Mo toxicity in ruminants.

Key words: Molybdenum transporter, *In silico* analysis, Buffalo.

INTRODUCTION

Molybdenum, an essential transition element in plants, animals and microorganisms, is required by several enzymes catalysing redox reactions in global carbon, nitrogen and sulphur metabolism (Rajagopalan 1991, Mendel *et al*. 2006). Although fifty different molybdoenzymes are found in nature, but in mammals, only three important molybdoenzymes namely xanthine oxidoreductase, aldehyde oxidase and sulphite oxidase are present where molybdenum serves as cofactor (MoCo) for their catalytic activity (Garattini *et al.* 2008, Zhang *et al.* 2008). The MoCo is synthesized in biological system by multistep biosynthetic pathway involving five enzymes MOCS1, MOCS2, MOCS3, Gephyrin and MOCOS (Schwarz 2005, Schwarz and Mendel 2006). After formation of MoCo it may be stored or utilized by insertion into apo-molybdoenzymes. From prokaryotes to eukaryotes molybdenum is essential as its absences causes lethality and it is required in traces because high doses of molybdenum may cause molybdenum toxicity to the living organisms. In bacteria molybdenum uptake depends on intracellular concentration of molybdenum (Turnlund 2002).

Molybdenum is present as molybdate ion in natural sources like soil and water and in this form, molybdenum is available for plants, animals and microorganisms. In bacteria several high affinity active transporters are present that helps to uptake molybdate from natural sources (Pau *et al.* 2002). They are mainly composed of ModA, ModB and ModC protein components and required ATP hydrolysis for their activity. Some bacteria can use molybdenum binding protein that can store molybdenum for future use. In plants molybdenum can transported via non-specific anion transporters (Hawkesford 2003), phosphate transporters (Baxter *et al.* 2007) and sulphate transporters (Hawkesford 2003); specifically group 5 of sulphate transporter which is reported as putative molybdenum transporter (MoT1).

[1]*Assistant Director, ARD, Institute of Animal Health & Veterinary Biologicals, Belgachia, Kolkata, India.*
[2]*Principal Scientist, Animal Biotechnology Centre, National Dairy Research Institute, Karnal, 132001, Haryana, India.*
**Corresponding author. e-mail: sanabtc@gmail.com*

Algae (Chlamydomonas) and plants (Arabidopsis) are only eukaryotes where first-time specific transporter for molybdenum MoT1 was identified which shared no sequence homology with animals (Tejada-Jiménez *et al.* 2007).

But little is known about molybdenum uptake, transport and utilization in animals. Recently one transporter system MFSD5, a member of MFS super family was reported as molybdenum transporter (HsMoT2) in human kidney cells (Nakanishi *et al.* 2013). It was reported that the HsMFSD5 shared sequence homology with algal Mo transporter (CrMoT2) (Tejada-Jimenez *et al.* 2011). MFS is the large and diverse group of single polypeptide secondary transporters including uniporters, symporters and antiporters that serve to transport different solutes and ions in all living organisms (Pao *et al.* 1998). These MFS members are highly conserved from prokaryotes to higher eukaryotes with common characteristic of having 12 transmembrane spanner topologies in most members from a primordial 6-TMS unit (Rubin *et al.* 1990). This evidence suggests that these MFS permeases are arose from intragenic duplication event throughout the evolution. Based on phylogenetic analysis MFS transporters are divided into 17 families (Saier 1994). Although MFSD5, a family member of MFS superfamily was considered to transport molybdenum in human cells so far, we did not gather sufficient information on molybdenum transporter in bovines, even no sequence data is available for buffalo MFSD5 as a putative BuMoT. In tropical countries ruminants are highly susceptible to molybdenum associated stress including molybdenum deficiency or molybdenum toxicity (Singh 2009). Low molybdenum availability in soil may provide indirect effect with loss of hair and hooves in cattle (Drögemüller *et al.* 2010) and xanthine caliculi in sheep (Askew 1958). In ruminants over consumption of molybdenum causes physiological copper deficiencies by formation of insoluble tetrathiomolybdate with severe symptoms like achromotrichia, anaemia, leg stiffness and infertility (Kubota 1978). A significant approach to identify a molybdenum transporter in bovines may pave a way to unravel molybdenum uptake and assimilation process in these species that could be exploited to manage molybdenum deficiency or toxicity in future. Bioinformatics analysis is the gateway for identification of Mo transporter gene in farm animals as well as facilitates to comprehend knowledge on physicochemical parameters, trans-membrane topology, predicted 3D model and functional annotation of the molybdenum transporter in animals.

In the present study we focus on identification of buffalo MoT by bioinformatics analysis followed by full length coding sequences (cds) cloning of buffalo MoT to obtain sequence information. Subsequently, we present sequence characterization of buffalo MoT by systematic bioinformatics approach to predict physicochemical parameter, 3D model and functional annotation along with bovine and human molybdenum transporter for better understanding of molybdenum transport mechanism in ruminants.

MATERIALS AND METHODS

Alignment of a Molybdenum transporter (MoT) sequence in mammals

The amino acid sequence of putative molybdenum transporter of *Chlamydomonas reinhardtii CrMoT* 2 (Accession No. XP_001693567), a member of MFS superfamily was taken from NCBI Database (Tejada-

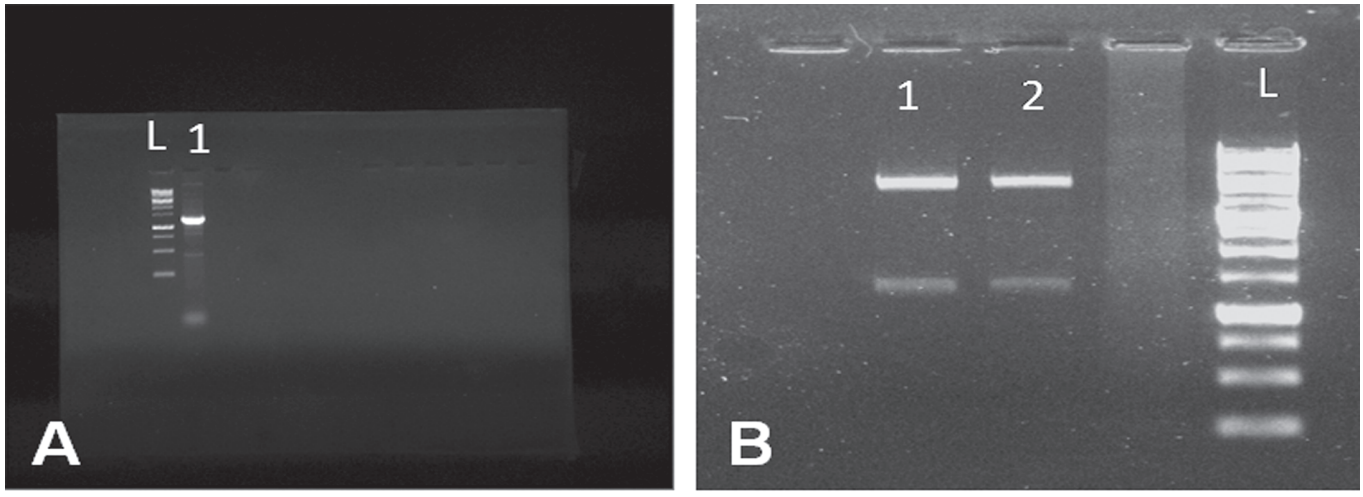

Fig. 1. recBuMoT plasmid (1.35 kb) by running in 1% Agarose gel electrophoresis (A, arrow) and double digestion of recMoT by NdeI and XhoI (B, arrow).

Jimenez *et al.* 2011). Alignment of proteins in different mammalian species under MFS superfamily with high similarity to CrMoT2 was performed by ClustalW method using ClustalW and ClustalX version 2.0 (Larkin *et al.* 2007).

Molecular cloning of buffalo MoT

Bovine MFSD5 was assumed as putative BoMoT from sequence conservation with CrMOT2 and recently characterized HsMoT2/MFSD5. Primer pairs for cloning buffalo MoT were designed in Primer 3 software using bovine MFSD5 sequence (Genbank ID:507921) which was retrieved from NCBI database (Benson *et al.* 2007).

Total RNA was isolated from buffalo mammary tissue by Trizol method. Full length first strand cDNA was prepared using Novagene cDNA synthesis kit. MoT gene was amplified by RT- PCR at optimized PCR conditions using MoT gene specific primer pairs. Restriction digestion of PCR product and PET 22b(+) vector was performed by NdeI and XhoI restriction endonucleases. Ligation was performed at 25°C and transformed the recombinant MoT into Top10 cells. Positive clones of recombinant MoT were screened by ampicillin resistance LB agar plate. To confirm the positive clones harboring MoT gene, recombinant plasmid was either digested by NdeI and XhoI or used for PCR amplification using gene specific MoT primers.

Sequencing of buffalo MoT

Recombinant Plasmid was isolated from positive clones by mdi plasmid isolation kit and used for sequence analysis using either MoT specific primer pairs or universal primers for PET 22b(+). Nucleotide sequence of BuMoT was confirmed by Nucleotide BLAST against bovine whole genome sequence.

Phylogenetic analysis of MoT protein in mammals

The amino acid sequence of BuMoT was obtained by translating the nucleotide sequence of BuMoT in Sequence Manipulation Suit. Alignment of MoT protein sequences in buffalo, cattle, horse, dog, human and mice were used for phylogenetic analysis of MoT protein in mammals (Hughes and Friedman 2007).

Prediction of physico-chemical characteristics of MoT protein

ProtParam (Gasteiger *et al* 2005) was used to study the physicochemical parameters of BoMoT and BuMoT, Parameters computed in ProtParam were molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index, and grand average of hydropathy (GRAVY).

Structural prediction of buffalo MoT

Amino acid sequences of bovine and buffalo MoT was submitted to structure prediction software Raptor X (Jian and Xu 2011). Raptor X was used to predict structure of whole protein.

The glycosylation sites were predicted from NetOGlyc, NetNGlyc and YinOYang tools, the phosphorylation sites were predicted from NetPhos 2.0 server and signal peptide was predicted by SignalP tool, provided by Centre for Biological Sequence Analysis, Technical University of Denmark (CBS DTU) (Gupta and Brunak 2002, Peterson *et al* 2011).

Prediction of transmembrane domain

Transmembrane domains of molybdenum transporter were predicted from Topred 0.01server using amino acid sequence of buffalo MoT (Heijne 1992, Claros *et al* 1994).

5'ATGCTGGTGACTGCTTACCTTGCTTTTGTGGTCCTCCTGGCCTCCTGCCTGGGGTTGGAGCTGTCAAGGTGCCGAGCTAAGCCCTCTGGA
AGGGCCTGCAGCGATCCCTCTTTCCTTCGGTTTCAACTGGACTTCTATCAGGTCTACTTCCTGGCCCTGGCAGCTGACTGGCTGCAGGCCCC
CTACCTCTACAAACTCTACCAGCATTACCACTTCCTGGAGGCACAAATTGCCATCCTCTACGTCTGCGGCCTTGCCCCCACCGTCCTCTTTGG
ACTGGTGGCTTCCTCCCTGGTGGATTGGCTGGGTCGCAAGAAATCTTGTGTCCTCTTCTCCCTCACTTACTCTCTCTGCTGCTTAACCAAACT
CTCCCGGGACTACTTTGTGCTGCTGGTGGGCCGAGCACTAGGTGGGCTATCTACAGCCCTGCTCTTCTCAGCCTTTGAGGCCTGGTATATCC
ATGAGCACCTGGAACGGCATGACTTCCCCGCCGAATGGATCCCGGCTACCTTTGCCCGAGCTGCCtTCTGGAACCATGTGCTGGCTGTAGC
GGCAGGTGTGGCCGCTGAGGCTGTGGCCTGCTGGATGGGCCTGGGGCCTGTAGCCCCCTTTGTGGCCGCCATCCCTCTCTTGGCCCTGGC
TGGGGCCTTGGCCCTTCATAACTGGGGAGAGAACTATGATCGGCAGCGTGCCTTCTCTAGGACCTGTGCTGGGGGCCTGCGCTGCCTCCT
GTCGGACCGTCGTGTGCTGCTGCTAGGCACCATCCAGGCCCTGTTTGAGAGTGTCGTTTTCATCTTTGTCTTCCTCTGGACACCTGTGCTGG
ACCCACATGGGGCTCCACTGGGCATCATCTTCTCCAGCTTCATGGCAGCCAGCCTACTCGGCTCTTCCCTGTACCGCATCGCTACCTCTAAGA
GGTACCACCTTCAGCCCATGCATCTACTCTCCCTCGCCGTCCTCATCGTCGTCTTCTCCCTCTTCATGTTGACTTTCTCTACCAGCCCAGGCCA
GGAGAGTCCAGTGGAATCTTTCATAGCCTTCCTCCTTATCGAGCTGGCCTGTGGACTGTACTTTCCCAGCATGAGCTTCCTGCGGAGAAAG
GTGATCCCTGAGACCGAGCAAGCTGGTGTCCTCAACTGGTTCCGGGTGCCCCTGCACTTATTGGCCTGCCTGGGGCTCCTGGTCCTCCATG
ACAGCGATCGCAAAaCGGGtACCCGGAATATGTTCAGCATCTGCTCCGCCGTCATGGTGGTGGCTCTGCTGGCAGTGGTGGGACTCTTCAC
CGTGGTCAGGCCCGACGGTGAGCTGCGGGTGCCCTCGCCCGCTGGGGAGCCCTACACTCCTGAGCTCTGA3'

Fig. 2. Full length cds sequence of recBuMoT.

MLVTAYLAFVVLLASCLGLELSRCRAKPSGRACSDPSFLRFQLDFYQVYFLALAADWLQAPYLYKLYQHYHFLEAQIAILYVCGLAPTVLFGL
VASSLVDWLGRKKSCVLFSLTYSLCCLTKLSRDYFVLLVGRALGGLSTALLFSAFEAWYIHEHLERHDFPAEWIPATFARAAFWNHVLAVAA
GVAAEAVACWMGLGPVAPFVAAIPLLALAGALALHNWGENYDRQRAFSRTCAGGLRCLLSDRRVLLLGTIQALFESVVFIFVFLWTPVLD
PHGAPLGIIFSSFMAASLLGSSLYRIATSKRYHLQPMHLLSLAVLIVVFSLFMLTFSTSPGQESPVESFIAFLLIELACGLYFPSMSFLRRKVIPET
EQAGVLNWFRVPLHLLACLGLLVLHDSDRKTGTRNMFSICSAVMVVALLAVVGLFTVVRPDGELRVPSPAGEPYTPEL

Fig. 3. Amino acid sequence of recBuMoT.

Functional annotation

Functional role of a protein is known from its conserved domain that share sequence homology with other orthologous proteins. Although we performed wet lab analysis of MFSD5 to delineate its role as molybdenum transporter we also used several bioinformatics tools to know its domain organization and related function. Functional annotation molybdenum transporter protein was performed by InterProscan (Zdobnov and Apweiler 2001), Pfam (Finn *et al.* 2010) and NCBI-CDD (Marchler-Bauer *et al.* 2011) database.

InterProScan is a tool that combines different protein signature recognition methods and provides functional analysis of proteins by classifying them into families and predicting the presence of domains and important sites.

Pfam is contains information about protein domains and families including domain architecture, annotation and family information of a protein using hidden Markov model. NCBI-CDD is a protein annotation resource that predicted functional domains by importing from number of external domains. It provides insights into sequence/structure/function relationships.

Additional information of MoT function was generated by using Proknow (Pal and Eisenberg 2005).

RESULTS AND DISCUSSION

Cloning and sequencing of BuMoT

The 1.5 kb amplified product of MoT/MFSD5 from buffalo mammary tissue was purified from PCR-reaction mixture using gel purification kit (Sigma) and cloned into a bacterial expression vector pET22b(+) followed by transformation. The positive clones were identified by restriction digestion and running of recMoT plasmid in 1% agarose gel electrophoresis (Fig. 1). The sequencing of full length cds of buffalo MoT was performed using cloned product which revealed 1353 bp sequence (Fig. 2). After translation in Expasy Tool, we obtained the amino acid sequence of BuMoT that was having 450 amino acids (Fig. 3).

MFSD5, a recently identified Mo transporter, is mainly involved in transportation of sugar molecules. In human and cattle, the protein is also 450 amino acids long suggesting a conserved protein. Buffalo is having 18 aa long signal sequence which indicate that it is a secretory protein.

Phylogenetic analysis

Phylogenetic analysis of Mo transporter in buffalo, cattle, human, horse, dog and mice revealed that buffalo MoT and bovine MoT share the same cluster (Fig. 4). It has been seen that Mo transporter from ruminant and non-ruminant form separate cluster whereas semi-ruminant species like horse form totally distant cluster. Among ruminants, cattle and buffalo shared the same clan whereas among non-ruminants human and mouse shared different clan. Other non-ruminant and carnivorous animals like dogs form the isolated cluster.

Predicted physic-chemical Parameter of buffalo MoT

Amino acid sequence of buffalo MoT was used to predict physicochemical properties of MoT proteins in buffalo and compared to cattle MoT and human MoT using ProtParam computation (Table 1). MoT protein from buffalo, cattle and human was shown to have 450 amino acids with average MW of 497KD. Cattle MoT is highly basic protein (pI 8.4) compared to buffalo (pI 7.9) and human MoT (pI 7.9). MoT protein is unstable, hydrophobic and insoluble irrespective of these three species.

Structural annotation of buffalo MoT

RaptorX structure prediction server used to predict 3D model of molybdenum transporter using lactase permease, glyceol 3 phosphate and as template in buffalo, bovine and human (Fig. 5).

From predicted 3D model of MoT in buffalo, human and bovine, it was found that the protein was believed to having two-fold pseudo symmetry between N and C terminal domain like glycerol 3 phosphate transporter and lactose permease (Xianjin *et al.* 2011, Chaptal *et al.* 2011).

Secondary structure of BuMoT was predicted from GOR4 software under ExPasy tools. BuMoT was shown to have 46.25% (209) alfa helix, 41.14% (189) random coil and 11.95% (54) extended strand (Garnier *et al.* 1996).

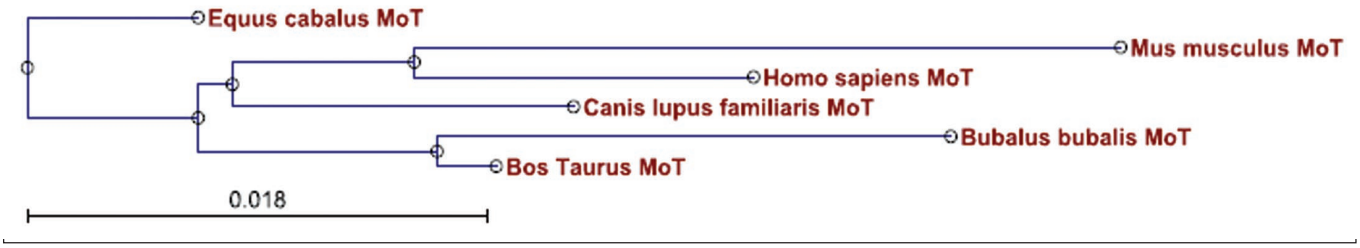

Fig. 4. Phylogenetic tree of MoT protein in mammals built by Mega 4.

Disulfind was used to predict disulfide bond in MoT proteins. No disulfide bond was found in BuMoT, BoMoT and HsMoT with high confidence of disulfide bond prediction state (Ceroni *et al.* 2006).

The glycosylation sites of BuMoT, BoMoT and HsMoT were predicted by using NetOGlyc, NetNGlyc 1.0 and YinOYang tools provided by CBS DTU (Fig.7, 8 and 9). NetOGlyc predicted potential O-glycosylation sites in MoT which were one for buffalo, two for cattle and one for human. In buffalo MoT no N glycosylation site was predicted by NetNGlyc 1.0; but for each case of cattle and human MoT, one N glycosylation site was predicted.

YinOYang 1.2 server predicted 8 O beta glycosylation sites in buMoT at residues 29 (Ser), 170 (Thr), 304 (Ser), 332 (Ser), 333 (Thr), 339 (Ser), 403 (Thr), and 447 (Thr) of which one site at 339 residues was predicted with high confidence level. In BoMoT also 8 O beta glycosylation sites were present at 29 (Ser), 170 (Thr), 304 (Ser), 332 (Ser), 333 (Thr), 339(Ser), 403(Thr) and 447 (Thr) residue of which 2 sites at 339 and 447 residues were predicted with high confidence level; whereas in HsMoT 6 O beta glycosylation sites at residues 170 (Thr), 304 (Ser), 332 (Ser), 333 (Thr), 339 (Ser), 403 (Thr) and serine reside at 339 position was predicted with high confidence level. Yin-Yang sites are serine/threonine residue that are O beta glycosylated and phosphorylated which may be changed by O-GlcNAc or phosphate groups in different species throughout the evolution. We studied on three mammals where common serine residue at 339 positions was predicted to have O beta glycosylation that might reflect as a conserved site in mammals (Fig. 6).

The results showed that MoT protein is highly glycosylated with inter species disparity in glycosylation pattern; even between same phylogenetic clusters.

Signal P predicted signal peptide sequence of 18 amino acids residues of MoT protein in buffalo for extracellular secretion (Fig. 7).

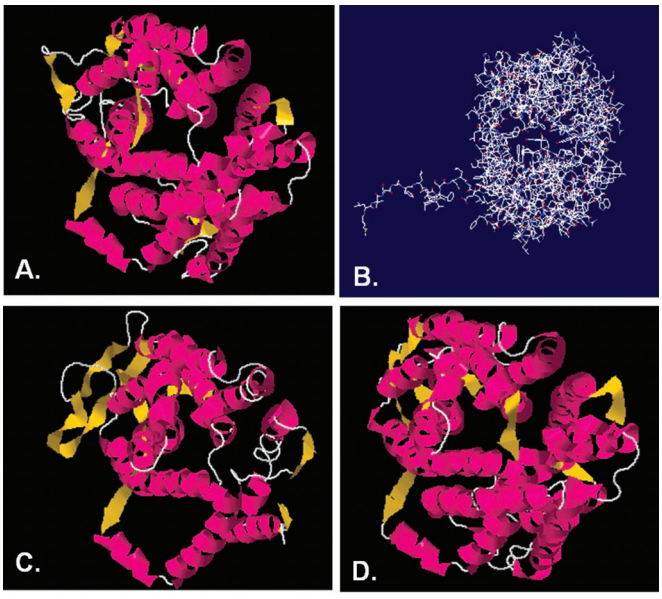

Fig. 5. 3D model of MoT in buffalo as predicted by RaptorX server.
A. Structure of whole BuMoT protein consisting of two pseudo-symmetrical N and C terminal domains;
B. Amino acid skeleton of whole BuMoT protein;
C and D: Two segments of BuMoT.

Functional signature of MoT/MFSD5

MFSD5, a member of MFS family was recently identified as molybdenum transporter in human cells, but its domain organization and functional annotation is not well understood. Using systematic bioinformatics approach, we gather sufficient information to annotate MoT protein in buffalo. NCBI-CDD, InterProScan and Pfam were used to get information about conserved domain and potential function of buffalo MoT. NCBI-CDD showed MoT protein is under MFS superfamily with MFS_1 multi-domain. This MFS is a large and diverse group of secondary transporters and antiporters that facilitate transport across cytoplasmic and internal membranes of variety of substrates including ions, sugar phosphates, drugs, neurotransmitter, nucleosides, amino acids and peptides. The majority of the MFS-transporter contains 12 transmembrane alfa helices connected by hydrophilic loops. The N and C terminal domains of this protein show weak similarity

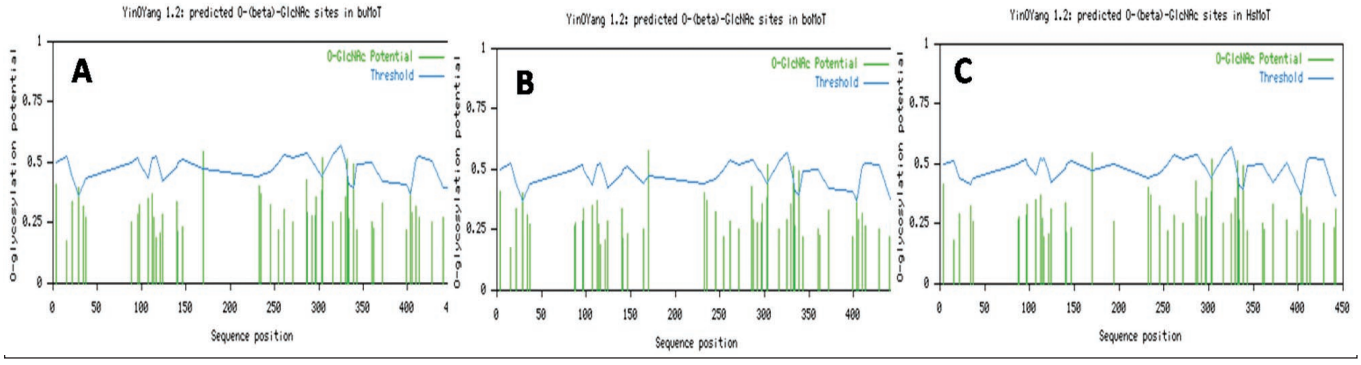

Fig. 6. O beta glycosylation of BuMoT (A), BoMoT (B) and HsMoT (C) as predicted by YinOYang 1.2 server.

which may arise from gene duplication or gene fusion event. Pfam described that the MoT protein is annotated with domain of unknown function (DUF791) under MFS clan (CL0015). This MFS clan contains 25 families in which 249360 domains are present (Pao *et al.* 1998). InterProScan combined different protein signature recognition methods that predicted MoT protein is under major facilitator superfamily domin, general substrate transporter (Fig. 8).

MFS was primarily believed to transport sugar molecules (Henderson and Maiden 1990). Exclusive studies on MFS gradually revealed that this expanded transporter family was believed to include drug efflux system, Krebs cycle metabolite, organophosphate: phosphate exchangers and oligosaccharide: H1 symport permeases. Reizer *et al.* (1994) noted that a mammalian phosphate: Na1 symporter is a distant member of this family; some MFS family members that are not still characterized were grouped under domain of unknown function (DUF) in Pfam. CDD and InterProScan predicted

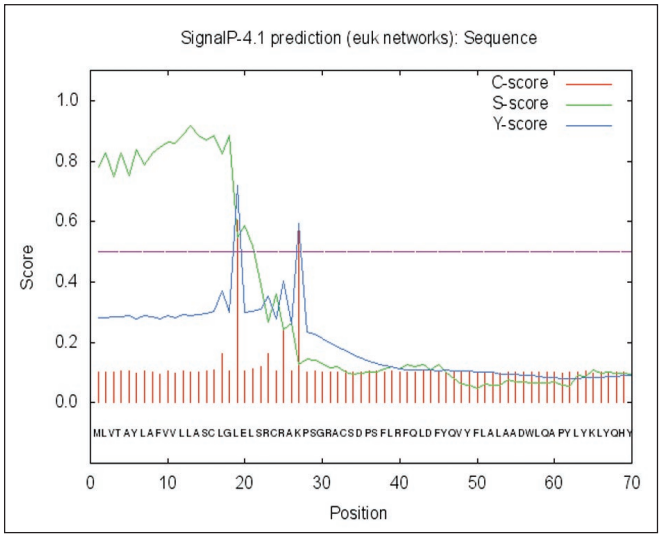

Fig. 7. Signal P predicted 18 amino acid long signal sequence in BuMoT.

MoT shares sequence conservation with MFS_1 family especially MFSD5, fifth member of sugar porter family. MFSD5 is also known as oligosaccharide: H1 symporter (OHS) family which constitutes six transporter proteins including LacY permease. The MoT protein shares structural homology with LacY permease having 12 TMS topological model (Calamia and Manoil 1990).

Weighted set of functions for a given sequence or structure were obtained using ProKnow knowledge database. Different protein features and algorithms were extracted from a given protein feature using DALI, DASEY, RIGOR, PROSITE, PSI BLAST, DIP programs. ProKnow metaserver predicted MoT protein is having kinase, dephospho-CoA kinase and transferase activity along with Mo and carbohydrate transport. MoT is also a structural component of ribosome where it regulates the translation. Additionally, it is having role in negative regulation of phosphorylation. After transportation, Mo is utilised by cells mainly by molybdoenzymes where Mo is present as catalytic co-factor named as Moco. This Moco is synthesized by five enzymes MOCS1, MOCS2, MOCS3, gephyrin and MOCOS (Schwarz 2005, Schwarz and Mendel 2006). Kinase like activity (transfer of phosphate group from ATP or high-energy donor molecules to specific substrate) of MoT is essential for carrying out signalling processes inside the cells that control utilization of Mo after transportation via Moco biosynthetic pathway. Similarly, dephospho-CoA kinase and transferase activity are essential for carrying out signalling processes. The MoT protein regulates translation (as it is also a structural component of ribosome) by synthesis regulating the synthesis of Moco biosynthetic pathway enzymes.

Transmembrane topology

Like other MFS permeases MoT protein showed common characteristic with having twelve trans membrane alfa helices connected by hydrophilic loops spanning across the membrane. This 12 trans membrane

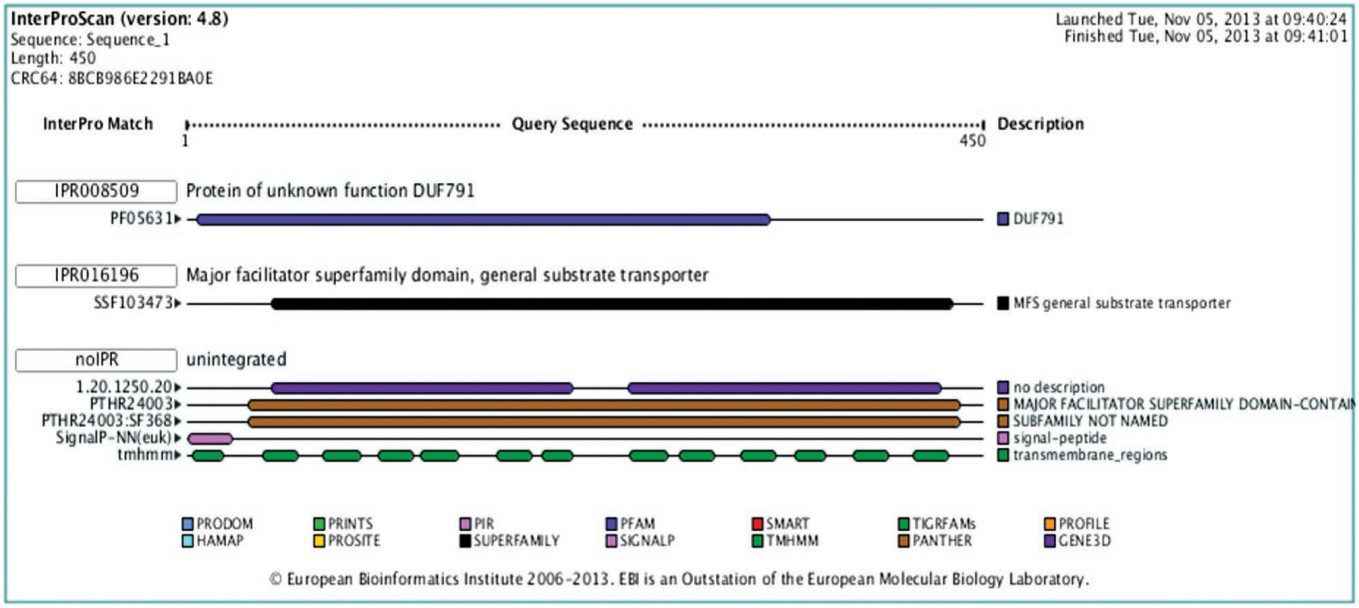

Fig. 8. Domain organization of BuMoT as predicted by InterProScan (version 4.8).

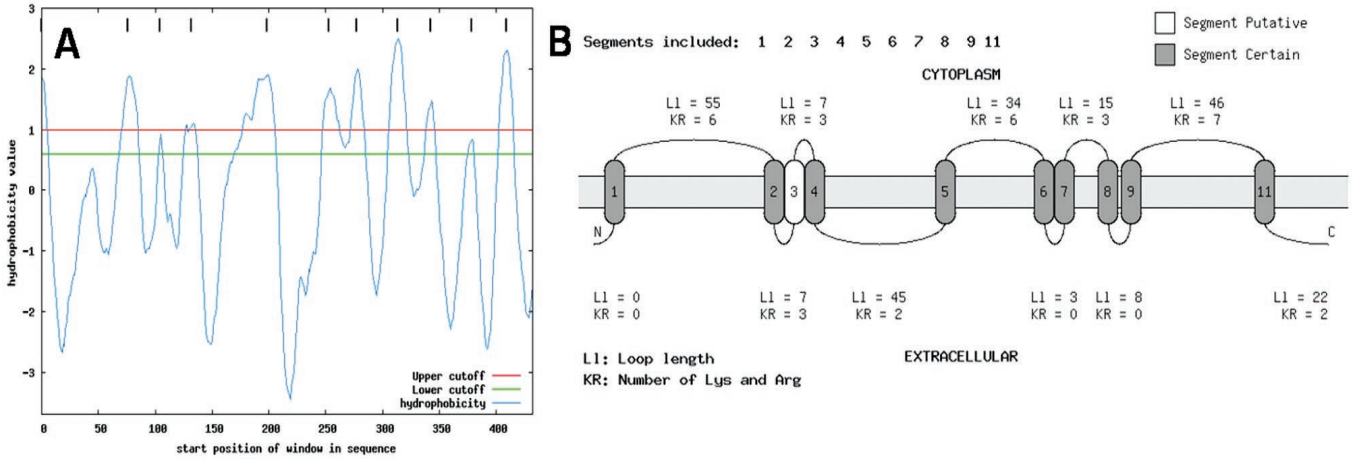

Fig. 9. Prediction of transmembrane domains of BuMoT by Topred 1.0 server.

spanner (TMS) topology of MoT protein might be generated from primordial 6 TMS unit as reflected by presence of two pseudo symmetrical domains (N and C terminal) each of which was consisting of six TMS in buffalo MoT 3D model. It was assumed that like MFS permeases MoT may arose from tandem intragenic duplication event (Rubin *et al.*1990). Trans membrane architecture of MoT protein was predicted by membrane protein topology prediction tool like Topred 0.01. Topred program predicts membrane topology based on hydrophobicity and the positive-inside rule. In buffalo eleven trans membrane segments were predicted to traverse membrane; each segment was connected by hydrophilic loops mainly containing lysine and arginine. N terminal site of MoT was predicted to present extra-cellularly whereas C terminal end was present inside the cytoplasm (Fig. 9). Tejada-Jiménez *et al.* (2011) predicted 11 transmembrane domains in CrMoT2 and 12 transmembrane domains in putative HsMoT which was conserved with other homologous protein (51-26% identity) in algae, plants and animals. We predicted 11 transmembrane domains in buffalo and bovine MoT of which third and tenth domains were putative in both cases.

CONCLUSION

For the first time, we report on identification, cloning and sequence characterization of Mo transporter in ruminants especially in buffalo. Buffalo Mo transporter is a 50 KD secretory protein that share sequence and structural homology with MFSD5, a member of MFS family. The N and C terminal domains of MoT are

Table 1. Comparison of Buffalo, Cattle and Human MoT.

Predicted Parameters	Buffalo MoT	Cattle MoT	Human MoT
No. of amino acids	450	450	450
Molecular Weight	49934.9 Dalton	49841.8 Dalton	49736.5 Dalton
Isoelectric point (pI)	7.94 (slightly positively charged)	8.38 (basic)	7.96 (slightly positively charged)
Instability index	45.9 (unstable)	45.56 (unstable)	46.83 (unstable)
Aliphatic index	116.99	116.0	115.36
GRAVY	0.680 (hydrophobic and insoluble)	0.670 (hydrophobic and insoluble)	0.651 (hydrophobic and insoluble)

pseudo-symmetrical. The protein is having 11 transmembrane alfa helices that traverse the cell membrane. Along with Mo transport, MoT protein was predicted to have additional role in cell signaling and protein synthesis that is further required for utilization of Mo via Moco biosynthetic pathway. Overall, this study would help to understand Mo homeostasis as well as pave a way to design inhibitors or mutant of MoT proteins for combating Mo associated stress including Mo deficiency and Mo toxicity in ruminants.

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